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Application of derm dotting in oral and maxillofacial surgery

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ABSTRACT

Purpose: Derm dotting is a new method of marking skin biopsies, and it is used by dermatopathologists to identify most diagnostic tissues on a microscopic slide. This method uses nail varnish to mark specific lesions and suspected section planes, or to orient skin resections. The nail varnish is resistant to different tissue processes, discernible while cutting the tissue block, and easily recognizable under the microscope. We examined the effectiveness of derm dotting on intraoral tissues.
Methods: We used the derm-dotting technique on the intraoral tissues of nine patients who underwent resection of a squamous carcinoma. We also tested this method on frozen sections.
Results: In all cases of resected tissue, the nail varnish was visible under gross examination, traceable while cutting the tissue block, and clearly visible in the definitive sections. The dots were preserved in the frozen sections, but they were lost in half of the decalcified tissues.
Conclusions: Derm dotting is an inexpensive, simple method that can replace the stitching technique used by surgeons to orient specimens. The stitches have to be removed by the pathologist, therefore possibly creating an artifact in the biopsy. The varnish dots or lines can be used to orient the specimen. In addition, the dots can easily mark suspect borders or areas of interest to be examined by the pathologist, using different colors, if desired. With derm dotting, the pathologist receives a more representative slide enabling a more accurate clinicopathological correlation.

1. Introduction

In modern medicine, biopsies are essential in making accurate treatment plans. Physicians rely on histological diagnosis to make decisions regarding surgery, chemotherapy, radiotherapy, or combinations of treatment. New technologies have been developed to help diagnose intraoral pathologies. One of the techniques for the earliest possible detection of oral precancerous lesions is direct oral microscopy, which involves obtaining biopsies according to colposcopic criteria [1,2]. When resecting a carcinoma, the surgeon has to communicate to the pathologist key information on the location, position, orientation, or width of the resection margins of the specimen. Surgeons currently use stitches to mark or orient the biopsy. When the biopsy arrives at the pathology department, technicians have to remove the stitches so that the microtome knife is not damaged, and the removed stitches leave an artifact with loss of tissue. Other surgeons do not mark the specimen, and they make only limited comments and drawings on the application form. Thus, new methods are needed to indicate to the pathologist the relevant parts of the tissue to examine and improve the accuracy of the histological report because a pathologist only examines a random 2% of the delivered tissue [3]. Therefore, it is very important that the proper areas to be examined are identified. The ideal method would be inexpensive, safe for the specimen, practical for the pathologist, and fast and easy for surgeons to apply.

Several different marking techniques have been proposed in the literature. Artist pigments are used to mark the resection borders of breast biopsy specimens [4]. In a cone biopsy of the ectocervix–endocervix transition zone, ink can be applied to areas of interest [5]. In pneumology, the bronchoscopist injects either carmine dye or methylene blue into the place of interest before using video-assisted thoracoscopic surgery (VATS) for resection [6–8].

In 2013, Haspeslagh et al. [9] described a new method called ex vivo dermoscopy with derm dotting. They discovered that nail

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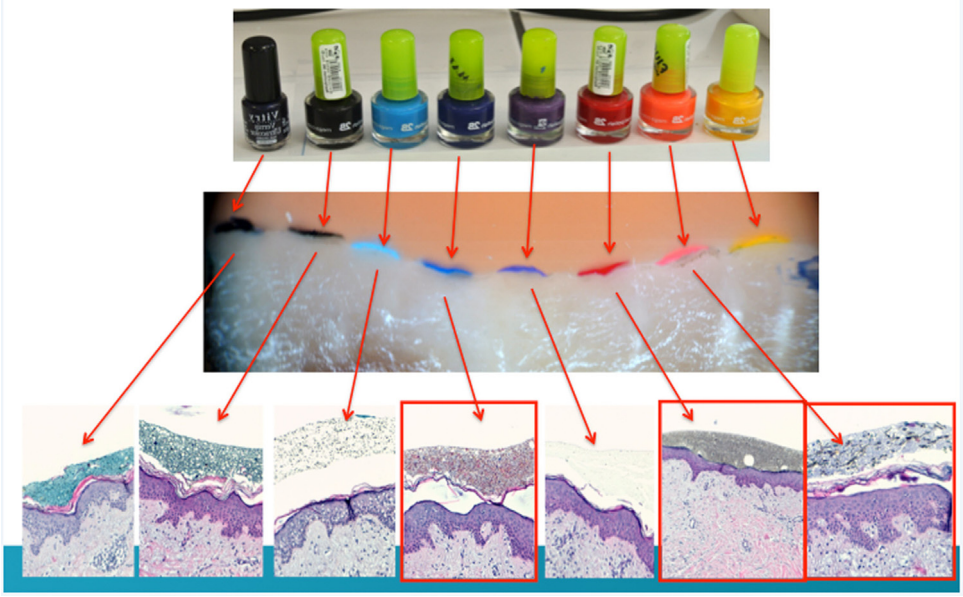

Fig. 1. Different types of colors can be distinguished by microscopy as different types of granular gray. In the middle, we see the different colors in the tissue block before processing (trimming).
Reproduced with permission from M. Haspeslagh.

varnish adheres to skin, is resistant to tissue processing, and can be detected under the microscope. Varnish dots can be used to mark specific areas to be examined by the pathologist or for orientation of the specimen. The nail varnish does not damage the tissue, and different types of nail varnish or different colors applied on the same biopsy can be distinguished on the tissue block and under the microscope. The different varnish colors are discernible under the microscope as different granular gray areas. In this study, we examined whether this derm-dotting technique is also applicable for lesion marking or orientation after resection of intraoral lesions. Our aim was to determine whether this nail varnish method can eventually replace the current, nonoptimal method of using stitches.

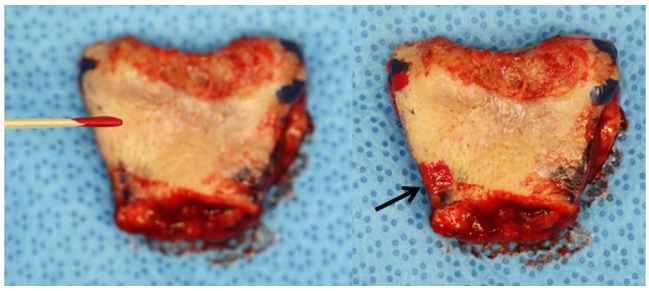

Fig. 2. Use of derm dotting on a lower lip specimen with squamous cell carcinoma. A combination of red and blue dots, applied with a toothpick, orients the specimen. A bigger red dot marks the place where the tumor free margin was clinically smallest.

2. Materials and methods

This experimental study was performed over a period of 3 months. The study complied with the principles laid down in the Declaration of Helsinki. The study was approved by the ethical committees of UZ-Leuven, Belgium (Ethical no: B322201319028). All nine subjects agreed and signed informed consent before being included in the study.

The method of derm dotting uses a simple, inexpensive nail varnish to mark the biopsy. In this study, we used the nail polish brand 2B Mega Colors. We used color 94 (Mat dark blue), 03 (American rose), 25 (Fluo yellow), 55 (Fluo red), and 04 (Cerise red). These colors were selected after testing many types of nail varnish on skin under a microscope (Fig. 1). Derm dotting was tested on 15 specimens of nine squamous carcinomas. Six out of the nine patients were male and the mean age was 67 years (Table 1). A primary resection with macroscopic free margins was performed in these patients. These resection specimens were placed on a surface next to the operation table. After completion of the surgery, the surgeon oriented the specimen using different colors of nail varnish dots for each side of the specimen. The small dots were marked on the tissue with a toothpick (Fig. 2), while the nailbrush was used for larger marks. Different colors were used to mark suspected resection borders or areas to be examined by the pathologist. After application, the nail varnish was left to dry for 3–5 min. Then, the specimen was placed in a closed biopsy tube (65 or 125 ml) with or without formol. After a maximum of 5 days, the pathologist checked whether the dots were still present on the specimen. In two patients, small fragments were taken for frozen sections, which were also marked with nail varnish for evaluation (Fig. 3). During this small study, different anatomic sites were examined, including the mucosa of the tongue, lip, cheek, uvula, mouth floor, and pharynx (Fig. 4). In addition, different types of tissue were examined, namely mucosa, fat tissue, submandibular, and sublingual gland tissue.

Table 1
Study population according to age and gender.

	Enrolled patients								
Number	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Gender	Male	Male	Female	Male	Female	Female	Male	Male	Male
Age (mean 67 years)	59	59	57	66	78	52	73	80	79

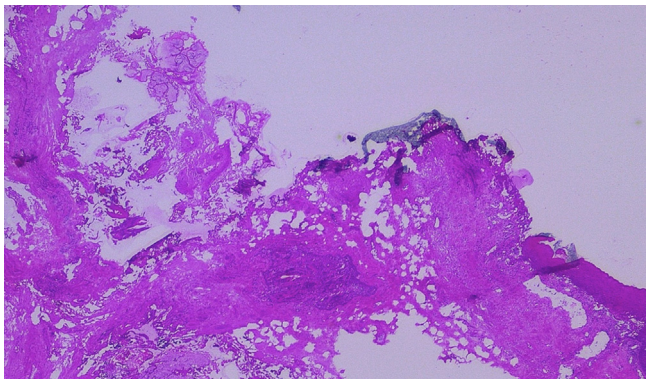

Fig. 3. Frozen section of a pharynx with a clearly visible gray granular layer of nail varnish, which was originally blue nail varnish.

When the specimen arrived at the pathology department, the technician trimmed through the tissue block until reaching the colored dot; then, the dotted sections were mounted on the microscopic slides. Specimens for definitive sections were divided into two groups. One group of specimens did not contain bone and underwent the normal histological processing as described (11 of 13 specimens). The other group included resection specimens with bone (two specimens), which were decalcified before the histological processing. On the definitive section, the pathologist traced the dots under the microscope, photographed the dotted areas, and made comments in the report.

3. Results

The results of our study are summarized in Table 2 for each type of section and specimen. Dots were placed in 15 specimens of squamous cell carcinoma. All of these dots were clearly visible in the laboratory before processing and during the cutting of the tissue block. The fluorescent orange and red varnishes were best seen in the tissue block. In the definitive sections without bone, all the dots were visible under the microscope and mentioned in the protocol by the pathologist, except for one case where the reporting pathologist did not mention the absence or presence of the marking dot (Fig. 4). The dots were also visible in the tissue block of the two frozen sections while cutting the slide and were also visible under the microscope (Fig. 3). In two of the four specimens that were decalcified before routine processing because of the presence of bone, the varnish mark was lost.

4. Discussion

This small study examined whether the method of derm dotting, described by Haspeslagh [9] in skin biopsies, was applicable in the field of maxillofacial surgery. In all 15 examined specimens, the varnish dots were clearly visible to the pathologist and technicians, thus enabling proper cutting and embedding of the biopsies. The different dots allowed a suitable orientation in the tissue blocks and during microscopic examination. The dotting method with varnish can replace the stitching technique or other techniques such as primitive drawings, or the use of alcian blue solution [10], colored gelatins [11,12], acrylic pigments [13], and Typp-ex fluid for marking the resection margins [14,15]. Indian ink is not very satisfactory due to the long drying time and the tendency of the ink to spread [16]. Acetone or Bouin's solutions have been used recently to aid in the drying of Indian ink [17–19]. In addition, other special color dyes in current use for marking section planes cannot be used to mark small areas because they also run out [20].

The new method of derm dotting is easy to use, and different parts of the resection specimen can be pointed out with different colors without spreading (Fig. 2). Proper orientation, section margins, or areas of interest of the biopsy can be easily communicated with the pathologist. In addition, from a material and cost point of view, stitches are considerably more expensive than a standard bottle of nail varnish. Furthermore, with this new method, there are no artifacts (no holes from stitches) as the nail varnish actually lies on the specimen without damaging it (Figs. 1, 3 and 4). The resins of the varnish responsible for adhesion to the intraoral tissue do not interfere with the cellular detail of the tissue [9]. The small varnish brush can be used to mark lines along a specific margin of the sections. After orientation and proper cutting of the specimen, the technician can cut the tissue block directly at the colored area, and therefore fewer slides have to be prepared and examined. The fluorescent orange and red varnishes are best seen in the tissue block. As with margin inks, blue and black nail varnish gave the best results under the microscope [11]. During the processing stage, the varnish color changes into different granular types of gray (Fig. 1), but an experienced eye can still easily distinguish between each shade of gray. This is similar to other tissue-marking dies where the colors are changed or even lost after routine processing and decalcification [21].

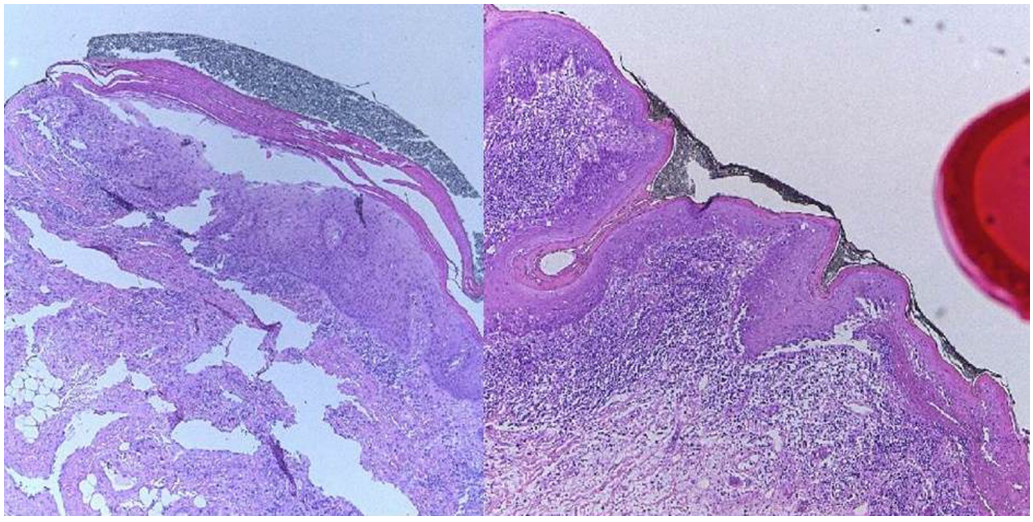

Fig. 4. Definitive sections of lip (a) and cheek (b) tissue with the granular gray nail varnish layer on top of the specimen without damage to the tissue.

Table 2
Types of sections and tissues with results if dot is microcopically visible or not after processing phase.

Sections	Dots microsopically visible after processing phase									
	Examined tissue	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Frozen sections	Uvula		Yes							
	Lip							Yes		
Standard sections	Tongue	Yes								Yes
	Lip							Yes		
	Cheek						Yes (bone)			
	Uvula		Yes							
	Mouth floor			Not reported	No (bone)	Yes (bone)				
	Pharynx		No (bone)							
	Fat tissue neck dissection	Yes							Yes	
	Glandula sublingualis	Yes								
	Glandula submandibularis								Yes	
	EXTRA: Bone in specimen	–	+	–	+	+	+	–	–	–

Finally, while examining the slide, the pathologist is able to control the right area internally, which allows a more confident and easier diagnosis and results in less demand for deeper cuts. With the varnish marks, the pathologist can easily reconstruct and orient the different slides of the specimen.

The method also seemed to work with frozen sections where one important area can be pointed out (Fig. 3 and Table 2). By contrast, the dot was lost in half the cases (two of four) when the definitive section was decalcified before processing because of the included bone in the resection specimen. The exact reason is unclear, but this study was conducted at a university hospital where different pathologist-trainees examined different cases. Not all of them might have looked for the dots with the same level of interest.

Finally, we believe this method can also be applied in other types of surgery. Therefore, we plan to test this method in non-oral surgically resected specimens.

5. Conclusion

The derm-dotting method with nail varnish is an inexpensive, easy marking system that can replace the use of stitches, dyes, or other creative techniques for orientation and marking of oral surgical specimens. We believe that this method can improve the quality of the clinicopathological communication between the surgeon and the pathologist. With this method, the pathologist is also more confident that the proper areas to be examined are identified. In addition, it could be useful to complement this new method with a standardized common color code system for communicating with the derm-dotting technique.

Conflict of interest

None of the authors has financial funding of conflict of interest.

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